sugars, oligonucleotides, nucleic acids, oligosaccharides, cells, cell fragments, tissue fragments, proteins and antibodies, but they are not limited to the named substances.

The invention is subsequently to be illustrated in more detail by means of embodiment examples and the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the accompanying drawings[.] :

Figure 1 shows the structural formula of benzopyrylium salt 2a[,] ;

Figure 2 shows the synthesis and structural formula of benzopyrylium salt 2b[,] ;

Figure 3 shows the synthesis and structural formula of trimethine OB11 (DY-630) [,] ;

Figure 4 shows the fluorescent spectra of OB15 (DY-635) in an aqueous solution and bound to bovine serum albumin (BSA) [,] ; and

Figure 5 shows the fluorescent excitation spectra of OB15 (DY-635) in an aqueous solution and bound to bovine serum albumin (BSA).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Embodiment Examples: claims

1. Instruction for the preparation of 11-(2,2-dimethylethyl)-9-methyl-1H,2H,3H,5H,6H,7H-pyrano[2,3-f]pyrido[3,2,1-ij]chinolin-12-ium tetrafluoroborate 2b (BS28), cf. Figure 2:

50ml of a 1.0 molar solution of methylmagnesiumbromide in dibutylether are added drop by drop to a cooled solution of 7.3g (0.0245mol) 11-(2,2-dimethylethyl)-1H,2H,3H,5H,6H,7H-pyrano[2,3-f] pyrido[3,2,1-ij]chinolin-9-on in 50ml ethylenglycol-dimethylether. The mixture was heated to a temperature of 40 degrees C for a time span of 30 minutes. After cooling down to 0 degrees C, 70ml of a saturated NH_4Cl solution and diluted hydrochloric acid were added for hydrolysis. The organic phase was separated and extracted using 4 x 10ml diethylether. The solvent was removed in a rotary evaporator and the oily residue was dissolved in 20ml pure acetic acid. The addition of 3ml HBF_4 (48 – 50%) and

the dilution with diethylether created a precipitant which is filtered out and recrystallized from pure acetic acid.

A yield of 3.35g (35%), melting point 175 – 180 degrees C. – ^{1}H NMR (400MHz, $CDCl_3$ + CF_3CO_2D): 1.43 (s, 9H), 1.90 (m, 2H), 2.06 (m, 2H), 2.67 (m, 2H), 2.92 (m, 2H), 3.35 (m, 2H), 3.57 (m, 2H), 3.95 (s, 3H), 6.90 (s, 1H), 7.58 (s, 1H): -$C_{20}H_{26}BF_4NO$ (383.24): calculated C 62.68, H 6.84, N 3.65, found C 63.06, H 6.72, N 3.48.

2. General instruction for the preparation of the non-symmetrical trimethines OB 11, OB 14, OB 15 and OB 20:

0.01mol of the corresponding 4-methyl-benzopyrylium-tetrafluoroboratee according to formula 2a (BS4) or 2b (BS28) (cf. Figure 1 and 2) and 0.01mol methylene-active N-heterocycle were dissolved in 20ml acetanhydride and after the addition of 2.0g of triethoxymethane and 5ml pyridine heated for about 10 minutes. The crude dye product was precipitated with 30ml of diethylether after the solution had cooled down to room temperature. The precipitate was filtered out and purified by means of column chromatography.

3. 1-(5-carboxypentyl)-3,3-dimethyl-2-[3-(7-N,N-diethylamino-2-(1,1-dimethylethyl)-4H-benzopyran-4-ylidene)-1-propenyl]-3H-indolium-5-sulfonate OB 11 (DY-630):

0.01mol of 2a and 0.01 mol of 1-(5-carboxypentyl)-2,3,3-trimethyl-3H-indolium-5-sulfonate were transformed according to the general specification 1, see Figure 3. Column chromatography: SiO_2, eluent ethanol. Yield of 3.2g (50%), melting point 280-282 degrees C. – ^{1}H NMR (400 MHz, DMSO-d6): 1.10 – 1.86 (m, 27H), 2.16 (m, 2H), 3.54 (m, 4H), 4.13 (m, 2H), 6.58 (d, 1H), 6.74 (s, 1H), 6.97 (s, 1H), 7.06 (d, 1H), 7.14 (d, 1H), 7.36 (d, 1H), 7.68 (d, 1H), 7.78 (s, 1H), 8.08 (d, 1H), 8.32 (t, 1H) – ^{13}C NMR (100 MHz, DMSO-d6): 12.30, 21.51, 24.32, 25.66, 26.56, 27.55, 34.07, 36.37, 43.72, 44.22, 48.87, 96,36, 99.40, 104.11, 109.85, 110.28, 112.48, 113.27, 119.66, 126.09, 140.23, 141.81, 145.59, 147.09, 162.14, 172.33, 174.64 – MS (FAB in dmba): 657 (M + Na^+), 635 (M + H^+), 391, 359, 258,

257 – $C_{36}H_{46}N_2O_6S$ (634.83): calculated C 68.11, H 7.30, N 4.41, found C 68.25, H7.33, N 4.39.

4. 1-(3-hydroxypropyl)-4-[3-(7-N,N-diethylamino-2-(1,1-dimethylethyl)-4H-benzopyran-4-ylidene)-1-propenyl]-chinolinium-tetrafluoroborate OB 14:

0.01mol of 2a and 0.01mol of 1-(3-hydroxypopyl)-4-methylchinolinium-iodide were transformed according to general specification 1. Column chromatography: SiO_2, eluent toluol/ethanol 1/1. Yield of 2.4g (42%), melting point 162-164 degrees C. – ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (t, 6H), 1.32 (s, 9H), 2.14 (m, 2H), 2.25 (s, 1H), 3.39 (q, 4H), 3.71 (m, 2H), 4.89 (m, 2H), 6.31 (d, 1H), 6.56 (s, 1H), 6.62 (m, 2H), 7.01 (d, 1H), 7.60 (t, 1H), 7.67 (d, 1H), 7.77 (d, 1H), 7.84 (t, 1H), 7.93 (d, 1H), 8.12 (t, 1H), 8.31 (d, 1H), 9.27 (d, 1H). –^{13}C NMR (100 MHz, $CDCl_3$): 12.52, 28.08, 32.01, 36.20, 44.61, 52.53, 57.52, 97.05, 97.94, 109.58, 109.94, 110.91, 111.77, 113.61, 117.72, 124.79, 125.38, 125.50, 127.13, 133.64, 137.96, 140.92, 142.20, 144.96, 150.87, 151.74, 155.40, 167.12 – MS (FAB in dmba): 483 (M^+) –$C_{32}H_{39}BF_4N_2O_2$ (570.48): calculated C 67.37, H 6.89, N4.91, found C 67.30, H 6.92, N 4.89.

5. 1-(5-carboxypentyl)-3,3-dimethyl-2-[3-(11-(2,2-dimethylethyl)-1H,2H,3H,5H,6H,7H-pyrano[2,3-f]pyrido[3,2,1-ij]chinoline-9-ylidene)-1-propenyl]-3H-indolium-5-sulfonate OB 15 (DY-635):

0.01mol of 2b and 0.01 mol of 1-(5-carboxypentyl)-2,3,3-trimethyl-3H-indolium-5-sulfonate were transformed according to the general specification 1.

Column chromatography: SiO_2, eluent ethanol. Yield of 2.9g (44%), melting point >300 degrees C. – ^{1}H NMR (250 MHz, DMSO-d6): 1.10 – 1.56 (m, 19H), 1.91 (m, 4H), 2.08 (m, 4H), 2.83 (m, 4H), 3.38 (m, 4H), 4.03 (m, 2H), 6.45 (d, 1H), 6.97 (s, 1H), 7.13 (d, 1H), 7.26 (d, 1H), 7.62 (d, 1H), 7.73 (s, 1H), 7.78 (s, 1H), 8.23 (t, 1H) – ^{13}C NMR (62 MHz, DMSO-d6): 19.40, 20.43, 24.86, 25.98, 26.61, 27.16, 27.76, 27.85, 28.94, 35.17, 36.71, 43.40, 48.45, 49.04, 49.63, 99.24, 102.90, 105.09, 109.69, 110.03, 112.96, 119.71, 121.85, 123.50, 139.89, 142.18, 144.84, 145.76, 148.56, 148.86, 151.59, 170.08, 171.37 – MS (ESI): 681 ($M + Na^+$),

659 ($M + H^+$), 352 – $C_{38}H_{46}N_2O_6S$ (658.12): calculated C 69.27, H 7.34, N 4.25, found C 69.20, H 7.37, N 4.29.

6. 1-(5-carboxypentyl)-4-[3-(7-N,N-diethylamino-2-(1,1-dimethylethyl)-4H-benzo-pyran-4-ylidene)-1-propenyl]-chinolinium-6-sulfonate OB 20:

0.01mol of 2a and 0.01mol 1-(5-carboxypentyl)-4-methyl-chinolinium-6-sulfonate were transformed according to general specification 1.

Column chromatography: SiO_2, eluent ethanol. Yield of 2.1g (35%), melting point >300 degrees C. – $C_{35}H_{42}N_2O_6S$ (618.76): calculated C 67.93, H 6.84, N 4.53, found C 67.73, H 6.93, N 4.29.

7. Preparation of the NHS ester of OB 11 (DY-630) with N-hydroxysuccinimide (NHS)/N,N'-dicylco-hexylcarbodiimide (DCC)

15mg OB 11 (DY-630), 14mg DCC and 4mg NHS were dissolved in 1ml dry DMF. After this, 1µl of thriethylamine were added. The reaction mixture was stirred for 24 hours at room temperature and then filtered. The solvent was then drawn off, the residue was washed with ether. This reaction was quantitative.

8. Preparation of the NHS ester of OB 15 (DY-635) with N-hydroxysuccinimide (NHS)/N,N'-dicylco-hexylcarbodiimide (DCC)

The process was analogous to example 7. This reaction also was quantitative.

9. Excitation and emission spectra of 1-(5-carboxypentyl)-3,3-dimethyl-2-[3-(11-(2,2-dimethylethyl)-1H,2H,3H,5H,6H,7H-pyrano[2,3-f]pyrido[3,2,1-ij]chinoline-9-ylidene)-1-propenyl]-3H-indolium-5-sulfonate OB 15 (DY-635)

The diagram in Figure 4 shows the emissions spectra and the diagram Figure 5 shows the excitation spectra of 1-(5-carboxypentyl)-3,3-dimethyl-2-[3-(11-(2,2-dimethylethyl)-1H,2H,3H,5H,6H,7H-pyrano [2,3-f] pyrido[3,2,1-ij]chinolin-9-ylidene)-1-propenyl]-3H-indolium-5-sulfonate when in water and when non-

covalently bound to bovine serum albumin (BSA), with the more intense spectrum being the one of the BSA conjugate. The concentration of both dye solutions was identical for these measurements.

10. General specification for the marking of proteins

The protein marking was done in a 50mM bicarbonate buffer (pH 9.0). A parent solution with 0.5mg reactive dye (for example OB 11-NHS-ester, M = 732 g * mol^{-1}) in 100µl DMF was created. The protein, for example avidine (M = 66000 g * mol^{-1}) was dissolved step by step in portions of 1mg in 200ml bicarbonate buffer; after this, varying volumes of the different and – if necessary – diluted dye parent solutions were added to the different protein aliquots. The reaction mixtures were then stirred for one to two hours at room temperature. The free dye was separated from the marked proteins by means of gel chromatography (Sephadex G25 medium, eluent PBS pH 7.2 22mM).

While the foregoing description and drawings represent the present invention, it will be obvious to those skilled in the art that various changes may be made therein without departing from the true spirit and scope of the present invention.